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Cooney and Emmerson, " Thermophilic Fungi ", (W.H. Freeman and Co.), 1964

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Description

This invention relates to an enzymatic detergent additive, which contains, as active components, a microbially produced lipase and a proteolytic enzyme, to a detergent composition comprising such enzymes, and to a washing process using such detergent.

The field comprising enzymatic additives in detergents has been rapidly growing during the last decades. Reference is made to e.g. the article "How Enzymes Got into Detergents", vol. 12, Developments in Industrial Microbiology, a publication of the Society for Industrial Microbiology, American Institute of Biological Sciences, Washington, D.C. 1971, by Claus Dambmann, Poul Holm, Villy Jensen and Mogens Hilmer Nielsen, and to P. N. Christensen, K. Thomsen and S. Branner: "Development of Detergent Enzymes", paper presented on 9 October 1986 at the 2nd World Conference on Detergents held in Montreux, Switzerland.

Proteolytic detergent additive is widely used in Europe, USA and Japan. In several countries, the majority of detergents, both powder and liquid, contain protease.

The use of lipase as a detergent additive is known. For a comprehensive review we refer to H. Andree et al.: "Lipases as Detergent Components", Journal of Applied Biochemistry, 2, 218-229 (1980). Further examples may be found in US 4,011,169 (column 4, line 65 to column 5, line 68), in GB 1,293,613 (page 2, lines 6-29) and in the paper by T. Fujii entitled "Washing of Oil Stains with Lipase" (in Japanese) given at the 16. Symposium on Washing, held in Tokyo on September 17-18, 1984.

Among the known lipases used as detergent additives, to the best of our knowledge the Fusarium oxysporum lipase has the best lipolytic characteristics, looked upon from a detergent application point of view, vide our European patent application with publication No. 0 130 064, especially the comparative table on page 27.

If the washing process is conducted at high temperature and high alkalinity, the majority of the fat containing dirt will be removed anyway. However, low or medium temperature washing processes (around 60°C and below) are now generally used, and at these low temperatures the known lipases are able to dissolve only a small part of the fat containing dirt.

Hitherto the efficiency of lipolytic detergent additives usually has been measured by washing of EMPA (Eidgenössische Materialprüfungs- und Versuchsanstalt, St. Gallen, Switzerland) swatches Nos. 101 (olive oil/cotton) and 102 (olive oil/wool) by adaptation of the procedure described in British patent No. 1,361,386 (especially pages 4 and 7) and US patent No. 3,723,250 (especially col. 15 - 19). In this way lipolytic cleaning efficiency can be expressed as the differential reflectance value Δ R. However, two more direct measures of the lipolytic action were used here. First, the weight of oil remaining on the textile was determined; this shows the combined effect of detergent and lipase. Second, the remaining oil was analyzed for oil (triglyceride) and decomposition products (mono- and diglyceride, and fatty acid), and the number of unhydrolyzed glyceride bonds in the oil was calculated; this shows more directly the effect of lipase. By use of these latter determinations it has been found that even the best known detergent lipase exhibits a lipolytic detergency effect which is open to improvement.

Furthermore, it is common knowledge that lipases, being proteins, are liable to attack by proteases, and as mentioned above, proteases are today contained in many detergents. There is no publication of a detergent lipase having satisfactory stability in the presence of protease. In fact, we have found that some known detergent lipases have poor stability in detergent solutions in the presence of commonly used detergent proteases.

Thus, a need exists for a lipolytic detergent additive which exhibits a considerably better lipolytic detergent efficiency at economically reasonable lipase activities in the washing solution and which is stable in detergent solutions containing detergent protease.

STATEMENT OF THE INVENTION

The first aspect of the invention provides an enzymatic detergent additive, which contains, as active components, a microbially produced lipase and a proteolytic enzyme, wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic activity is between about 0.5 and about 3.0 ANSON units/g of additive.

In another aspect, the invention provides an enzymatic detergent additive, which contains, as active components, a microbially produced lipase and a proteolytic enzyme, wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic activity is an alkaline Bacillus protease. Preferably, the proteolytic enzyme is manufactured microbially from Bacillus licheniformis or is manufactured according to US Patent No. 3,723,250.

The well-known Anson hemoglobin method for proteolytic activity is described in Journal of General Physiology, 22, 79-89 (1959).

Further aspects of the invention provide a detergent composition comprising a microbially produced lipase and a proteolytic enzyme wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic activity is between about 0.0005 and 0.15 ANSON units/g of detergent, and a detergent composition comprising a microbially produced lipase and a proteolytic enzyme, wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic enzyme is an alkaline Bacillus protease. Preferably, the proteolytic enzyme is manufactured microbially from Bacillus licheniformis or is manufactured according to US Patent No. 3,723,250.

Finally, the invention provides a washing process using the above detergent composition at a pH between 7 and 12.

The detergent lipases contained in the detergent additive of the invention show a superior detergency compared to previously known detergent lipases. Further, the lipases used in the detergent additive of the invention are stable in detergent solution in the presence of commonly used detergent proteases, in contrast to known detergent lipases.

It is described in Japanese unexamined patent publication No. 48-62990 that Humicola lanuginosa is a lipase producer. However, this Japanese patent publication fails to suggest that the H. lanuginosa lipase is suited as an active component in an enzymatic detergent additive. On the contrary, it appears from fig. 1 in the Japanese patent publication that the pH-optimum of H. lanuginosa lipase is around 8, and that the activity declines sharply when the pH value increases above 8. Thus, it would be expected that this lipase is unsuited as a detergent additive, as the pH in washing solution is usually far above 8. Surprisingly, however, we have found that the H. lanuginosa lipase has a pH optimum far above 8, vide Example 1 later in this specification.

Also, it is described in Current Science, August 5, 1981, Vol. 50, No. 15, page 680 that H. lanuginosa lipase can be used in dry cleaning. As the pH optimum, which exclusively relates to aqueous media, is of no significance whatsoever in relation to a dry cleaning lipase, this statement is not relevant to a possible suitability of the H. lanuginosa lipase as a lipolytic detergent additive.

Furthermore, it appears from Agr.Biol.Chem. 37 (11), p. 2488 (1973) that H. lanuginosa lipase is strongly inhibited by addition of certain anionic surfactants. However, we have found that surprisingly H. lanuginosa lipase is excellently compatible with LAS, a commonly used anionic surfactant.

DETAILED DESCRIPTION OF THE INVENTION

Lipase-producing microorganisms

Lipases of the invention are obtainable from strains of thermophilic Humicola sp., also classified as thermophilic Thermomyces sp.. Examples of suitable strains are H. lanuginosa (Griffon and Maublanc) Bunce, H. stellata Bunce, H. grisea var. thermoidea, Cooney & Emerson, H. insolens, Cooney & Emerson, Thermomyces ibadanensis, Apinis & Eggins, H. hyalothermophila Moubasher, Mazen and Abdel-Hafez, H. grisea var. indica Subrahmanyam, H. brevis var. thermoidea Subrahmanyam and Thirumalachar and H. brevispora Subrahmanyam and Thirumalachar.

In a specially preferred embodiment of the enzymatic detergent additive according to the invention the lipase is producible from H. lanuginosa (Griffon and Maublanc) Bunce, H. brevispora Subrahmanyam and Thirumalachar, H. brevis var. thermoidea Subrahmanyam and Thirumalachar or H. insolens Cooney & Emerson.

H. lanuginosa has also been described under the synonyms Thermomyces lanuginosus Tsiklinsky, Sepedonium lanuginosum Griffon and Maublanc, Sepedonium thermaphilum cyclosporum and S. thermaphilum ovosporum Velich, Acremoniella sp. Rege, Acremoniella thermophila Curzi and Monotospora lanuginosa (Griffon and Maublanc) Mason.

Moreover, the species Scytalidium thermophilum (Cooney & Emerson) Austwick was by Hedger (1975, The ecology of thermophilic fungi in Indonesia. In biodegradation et Humification. Rapport due ler Colloque International - Nancy 1974 (ed. G. Kilbertius, O. Reisinger, A. Mourey & J. A. Cancela Da Fonseca), Sarreguemines: Pierron Editeur - 57206) considered as belonging to Humicola insolens.

In a particularly preferred embodiment the lipase is producible from one of the following strains:

taxonomic designation	internal No.	deposit No.	deposit date
H. lanuginosa	A 1231	DSM 3819	13 Aug 1986
H. lanuginosa	H 126	DSM 4109	4 May 1987
H. brevispora	A 2121	DSM 4110	4 May 1987
H. brevis var. thermoidea	A 2106	DSM 4111	4 May 1987
H. insolens	C 579	DSM 1800	1 Oct 1981

DSM indicates Deutsche Sammlung von Mikroorganismen. The strains have been deposited under the terms of the Budapest Treaty.

Lipase for use in the invention may be produced by aerobic cultivation of one of the above strains according to principles known in the art, e.g. as the examples given later.

Immunochemical characterization of lipases

It is to be understood that lipases produced by genetic engineering on the basis of Humicola sp. and with active centers identical to the active centers of the lipases producible from Humicola sp. are also within the scope of this invention. The lipases for inclusion in the detergent additive of the invention are those which cross-react immunologically with (are antigenically identical or partially particularly with the lipase from one of the above-mentioned species, particularly H. lanuginosa and especially from one of the above-mentioned strains, notably DSM 3819 and DSM 4109.

The identity (cross-reaction) tests can be performed by the well-known Ouchterlony double immunodiffusion procedure or by tandem crossed immunoelectrophoresis according to N.H. Axelsen: Handbook of Immunoprecipitation-in-Gel Techniques (Blackwell Scientific Publication, 1983), Chapters 5 and 14. The terms "antigenic identity" and "partial antigenic identity" are described in the same book, Chapters 5, 19 and 20.

Using monospecific rabbit antiserum raised against purified lipase from DSM 4109, we found that the lipases from strains DSM 3819, DSM 4109, DSM 4110 and DSM 4111 are all antigenically identical by both of the above-mentioned methods. Production of antiserum is described in N.H. Axelsen's book, Chapter 41. Purification of lipase is described in W-H Liu, Agr. Biol. Chem., 37(1), 157-163 (1973); however, we found that the column chromatography may be more conveniently performed by use of: DEAE-sepharose (anion exchange chromatography), phenyl sepharose (hydrophobic interaction chromatography), followed by gel filtration on TSK G3000SW.

Enzymechemical characterization of lipases

The pH dependence of the activity was determined by a traditional method, using tributyrine as substrate at 30 °C in a pH stat and with gum arabic as emulsifier. The activity at various pH was found from alkali consumption versus time.

pH dependence was also checked with a more realistic substrate, viz. olive oil adsorbed on PVC (according to US patent 4,284,719).

pH-activity curves for lipase from H. lanuginosa DSM 3819 are shown in fig. 1 (tributyrine) and fig. 2 (olive oil/PVC). The curves for DSM 4109, DSM 4110 and DSM 4111 were very similar, showing optimum at pH 10.0 - 10.5 by both methods. pH-activity curves for lipase from H. insolens DSM 1800 are shown in fig. 3 (tributyrine) and fig. 4 (olive oil/PVC).

Isoelectric focusing was performed on the five lipases, followed by a tributyrine overlayer to detect lipase activity. It was found that DSM 3819, DSM 4109, DSM 4110 and DSM 4111 all have lipase activities with pI around 4.5, while DSM 1800 has the main part of its lipase activity with pI around 9.0 - 9.5 and only a minute amount of the lipase activity with pI around 4.5.

Detergent additive

In a preferred embodiment, the enzymatic detergent additive according to the invention is provided as a non-dusting granulate or as a liquid. These are suitable for use in powder detergents and liquid detergents, respectively. Granulates can be produced in several different ways. Reference can be made to GB patent No. 1,362,365 which describes the production of enzyme containing granulates used as detergent additives by means of an apparatus comprising an extruder and a spheronizer (sold as MARUMERIZER®), and to US patent No. 4,106,991 which describes the production of enzyme containing granulates used as detergent

additives by means of a drum granulator.

In the case of a liquid formulation, storage stability tends to be unsatisfactory, and a liquid with an enzyme stabilizer is therefore preferred. The stabilizer can be propylene glycol or other agents known as stabilizers for enzyme solutions. As will be shown later in this specification, a straight aqueous solution of the lipase of the invention has poor storage stability, but this can be remarkably improved by the inclusion of stabilizers, e.g. propylene glycol.

In a specially preferred embodiment of the enzymatic detergent additive according to the invention, the lipase activity is above about 10,000 LU/g of additive. Lipase Unit (LU) will be defined later in this specification. In this manner, a convenient lipase activity is generated in the washing solution when the detergent additive is added to the detergent in an amount of 0.1 to 5.0 g/100 g of detergent, and when the detergent is added to the washing solution in an amount of 0.5 - 20 g of detergent/l of washing solution.

Proteolytic enzyme

In the detergent additive of the invention, alkaline Bacillus proteases are preferred due to their well-known efficiency as detergent proteases. As such enzymes the proteolytic enzyme manufactured microbially by cultivation of Bacillus licheniformis, or the proteolytic enzymes manufactured according to US patent No. 3,723,250, can be used. Such proteolytic enzymes are available from Novo Nordisk A/S, under the trade marks Alcalase®, Savinase® and Esperase®. The enzymatic detergent additive of the invention can be prepared either by mixing a previously prepared granulate of proteinase with a previously prepared granulate of lipase, or by mixing a concentrate of proteinase with a concentrate of lipase and then introducing this mixture into a granulating device, together with the usual granulating aids.

A convenient proteolytic activity is generated in the washing solution when the detergent additive is added to the detergent in an amount of 0.2 - 2 g/100 g of detergent, and when the detergent is added to the washing solution in an amount of 0.5 - 20 g of detergent/l of washing solution.

The enzymatic detergent additive of the invention may also contain other detergent enzymes apart from lipase and protease, such as amylase or cellulase.

Detergent

In accordance with the previously indicated embodiments of the additive according to the invention, the detergent according to the invention may be a powder or a liquid and may optionally include other detergent enzymes, such as

amylase or cellulase, either in the same additive or as separate additives.

In a specially preferred embodiment of the detergent according to the invention, the detergent contains the enzymatic detergent additive according to the invention in an amount of between 0.1 and 5% w/w, more preferably in an amount of 0.2 - 2% w/w. In this manner, a reasonable balance between enzyme action and the action of the other detergent ingredients is generated.

The detergent is typically used in concentrations of 0.5 - 20 g/l of washing solution, and suitable lipase activity in the washing solution is 1,000 - 10,000 LU/l, more preferably 1,000 - 5,000 LU/l. Accordingly, in a preferred embodiment the lipase activity in the detergent is 50 - 20,000 LU/g, more preferably 50 - 10,000 LU/g, still more preferably 250 - 2,000 LU/g and most preferably 500 - 2,000 LU/g of detergent.

As mentioned above, the preferred lipase in the additive is above 10,000 LU/g, and this is added to the detergents in amounts of preferably 0.1 - 5% w/w and more preferably 0.2 - 2% w/w. Accordingly in another preferred embodiment the lipase activity in the detergent is 10 - 500 LU/g, more preferably 20 - 200 LU/g of detergent.

In accordance with the above-mentioned preferred ranges for protease activity in the additive and for the amount of additive in the detergent, we prefer a protease activity in the detergent of 0.0005 - 0.15 AU/g, more preferably 0.001 - 0.060 AU/g, still more preferably 0.003 - 0.025 AU/g and most preferably 0.006 - 0.010 AU/g of detergent.

In a specially preferred embodiment of the detergent according to this invention, the surface active material comprises 30-100% anionic and 0-70% non-ionic surfactant, most preferably 50-100% anionic and 0-50% non-ionic surfactant. Detergency of lipase of this invention is specially pronounced in detergents with a high content of anionics, such as LAS (linear alkyl benzene sulfonate).

Washing method

In a specially preferred embodiment of the washing process according to the invention, the washing solution contains the detergent according to the invention in an amount of between 0.5 and 20 g/l of washing solution. In this manner, a convenient lipase activity is generated in the washing solution, i.e. typically between 1,000 and 10,000 LU/l of washing solution, preferably between 1,000 and 5,000 LU/l of washing solution.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 - 4 show pH-activity curves, figs. 1 - 2 are for DSM 3819 lipase, and figs 3 - 4 for DSM 1800 lipase. Figs. 1 and 3 are by the tributyrine method, and figs. 2 and 4 by the olive oil/PVC method.

EXAMPLES

Lipase activity

The method is based on hydrolysis of tributyrin in a pH-stat. 1 LU (Lipase Unit) is the amount of enzyme which liberates 1 μmol titratable butyric acid per minute at 30°C, pH 7.0 with gum arabic as an emulsifier. Further details are given in Novo analytical Method AF 95/5, available on request.

EXAMPLE 1

Lipase from H. lanuginosa DSM 3819

Each of fourty 500 ml shake flasks with 200 ml PL-IC medium (composition indicated below) in each were inoculated with 0.2 ml of a spore suspension prepared on the basis of slants with H. lanuginosa DSM 3819 grown on YPG-agar (composition indicated below) for 5 days at 45°C. The thus inoculated shake flasks were shaken 3 days at 45°C at 240 rpm. At this stage the lipase activity of the accumulated broth (6.7 litres) was 104 LU/ml. The cells were removed by centrifugation at 4000 rpm for 25 minutes. 5.9 litres of supernatant was obtained. The supernatant was filtered through a 10 μ nylon filter cloth prior to 8 x concentration by ultrafiltration on Pellicon UF Cassette system (membranes with NMWL of 10,000, NMWL being an abbreviation of nominal molecular weight limit).

The UF-concentrate (final volume of 740 ml) was converted to a crude powder by freeze-drying. This crude powder exhibited a lipase activity of 13,310 LU/g.

Composition of YPG-agar was as follows:

Yeast extract, Difco	4 g/l
Glucose	15 g/l
K_2HPO_4	1 g/l
$MgSO_4$, $7H_2O$	0.5 g/l
Agar	20 g/l
Autoclaved at 121°C for 40 minutes.	

Composition of PL-Ic medium was as follows:

Peptone	15 g/l
Tween-80	18 g/l
$MgSO_4$, $7H_2O$	2 g/l
$CaCl_2$, $2H_2O$	0.1 g/l
Nalco-10	2 g/l
pH before autoclaving	6.0
Autoclaved at 121°C for 40 minutes.	

EXAMPLE 2

Lipase from other Humicola strains

Strain DSM 4111 was fermented on medium PL-Ic, strain DSM 4109 on medium GT, strain DSM 4110 on medium GTS-I, and strain DSM 1800 on medium LR-8ST, essentially as described in Example 1.

Composition of fermentation media:				
		GT	GTS-I	LR-8ST
Yeast extract (65% dry matter)	g/l	22.5	15	0
Pharmamedia	-	0	0	50
Tween 80	-	18	5	5
Span 80	-	0	5	5
$MgSO_4,7H_2O$	-	2	2	0.5
$CaCl_2,2H_2O$	-	0.1	0.1	0
K_2HPO_4	-	0	0	5
$NaNO_3$	-	0	0	1
Nalco-10	-	5	2	0
pH before autoclaving		~6.5	~6.0	~7.0

Recovery from the culture broths was performed essentially as described for DSM 3819 in Example 1. In the instance of lipase from DSM 4109, an additional purification step was performed prior to freeze-drying: the UF-Concentrate was precipitated with acetone whereafter the precipitate was redissolved in water and freeze-dried. The resulting freeze-dried powders exhibited the following lipase activities:

Strain	DSM 4111	DSM 4109	DSM 4110	DSM 1800
LU/g	32,000	211,000	6,600	1,800

Washing method

The test material employed for washing trials was cotton fabric (with a surface weight corresponding to around 1.2 g/50 cm^2) impregnated with olive oil (Sigma 0-1500). The swatches were produced simply by dropping 50 or 85 µl (as indicated) of olive oil heated to 50-60 °C on the centre of each test swatch (7 x 7 cm) by means of a micropipette. After oil application, the swatches were aged at room temperature for about 2 days.

The lipase preparations from Examples 1 and 2 were used, each identified by the strain number.

Also, as a comparison, a lipolytic powder on the basis of Fusarium oxysporum, obtained as described in Example 23 in European patent publication No. 0 130 064, and representing the most efficient known lipolytic detergent additive, was used. The activity of the Fusarium oxysporum lipase preparation was 90,000 LU/g.

The lipases were evaluated in washing tests in a Terg-O-Tometer test washing machine. The Terg-O-Tometer test washing machine is described in Jay C. Harris, Detergency evaluation and testing, Interscience Publishers Ltd., 1954, pages 60-61.

The washing trials were carried out under the following conditions:

Agitation	100 rpm
Water hardness	18 °German hardness (tap water) unless otherwise noted
Cloth/liquid ratio	7 swatches/1000 ml
Rinsing	15 min in running tap water

The amount of oil contained in 7 swatches with 85 µl each is approx. 535 mg (density 0.90).

After rinsing, the swatches were air dried. The residual oil content in the swatches was determined by Soxhlet extraction with n-hexane for 5 hours followed by gravimetric determination of residual matter.

The composition of the residual oil was analyzed by the TLC-FID (TLC/FID is an abbreviation for thin layer chromatography/flame ionization detector, the method being described in Lipids, vol. 18, No. 10 (1983), page 732) method using a Iatroscan TH-10 (Iatron Lab. Inc., Tokyo) combined with a Chromatocorder II (System Instruments Co., Ltd., Tokyo) computing integrator under the following conditions:

Stationary phase	Chromarod S-II (Iatron)
Mobile phase	Hexane/chloroform/acetic acid (60:50:2 v/v/v)
Hydrogen flow rate	160 ml/min
Air flow rate	2000 ml/min
Scan speed	30 sec per scan

Samples for the TLC-FID analysis were prepared as follows. After gravimetrical determination of residual matter the dried extract was redissolved in 20 ml of hexane and 5 ml of an internal standard (lithocholic acid, 12.5 mg/ml) dissolved in ethanol was added. 1 µl of sample was used for each analysis.

Based on standard curves for triolein, diolein, monoolein, and oleic acid the relative composition (% w/w) of the residual oil was calculated.

The number of unhydrolyzed glyceride bonds in the residual oil was calculated using the following formula:

$$n = 10 \times M \left(\frac{3}{885} \times X_{TG} + \frac{2}{621} \times X_{DG} + \frac{1}{357} \times X_{MG}\right) \quad (\mu \text{ mole})$$

where X_{TG} is the percentage of triglyceride (% w/w)

X_{DG} is the percentage diglyceride (% w/w)

X_{MG} is the percentage monoglyceride (% w/w)

M is the residual amount of oil (mg)

885, 621, and 357 are the mole weights for triolein, diolein, and monoolein, respectively.

EXAMPLE 3

Effect of washing temperature

This example demonstrates the effect of the Humicola lanuginosa lipase (DSM 3819) in an anionic detergent at different wash temperatures.

Detergent composition:	LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Washing time:	20 min.
Lipase dosage:	3000 LU/l
pH:	9.5
Soiling:	85 µl olive oil

LAS is a linear alkyl benzene sulfonate (Nansa HS80/S, Albright & Wilson), an anionic surfactant.

Temp.		Without lipase	Fusarium oxysporum	Humicola lanuginosa
30 °C	Residual oil (mg)	185	187	165
	n (µ mole)	590	571	360
50 °C	Residual oil (mg)	187	192	157
	n (µ mole)	606	628	432

EXAMPLE 4

Effect of washing time

In this example the effect of the Humicola lanuginosa lipase (DSM 3819) is demonstrated using different washing times.

Detergent composition:	LAS (0.5 g/l), Na_2CO_3(1.0 g/l)
Temperature:	30 °C
Lipase dosage:	3000 LU/l
pH (initial):	9.5
Soiling:	85 µl olive oil

Washing time (min.)		Without lipase	Fusarium oxysporum	Humicola lanuginosa
20	Residual oil (mg)	185	187	165
	n (µ mole)	590	571	360
40	Residual oil (mg)	177	167	128
	n (µ mole)	568	526	246
60	Residual oil (mg)	141	147	93
	n (µ mole)	465	454	153
90	Residual oil (mg)	139	135	78
	n (µ mole)	431	419	111

EXAMPLE 5

Effect of water hardness on washing

This example shows the influence of water hardness on the detergency of Humicola lanuginosa lipase (DSM 3819). The hardness (°GH = °German hardness) was adjusted by mixing tap water and distilled water.

Detergent composition:	LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Temperature:	30 °C
Washing time:	20 min.
Lipase dosage:	3000 LU/l
pH (initial):	9.5
Soiling:	85 µl olive oil

Hardness °GH		Without lipase	Fusarium oxysporum	Humicola lanuginosa
0	Residual oil (mg) n (μ mole)	254 820	244 752	242 627
6	Residual oil (mg) n (μ mole)	210 670	192 595	173 415
12	Residual oil (mg) n (μ mole)	182 579	179 548	170 405
18	Residual oil (mg) n (μ mole)	185 590	187 571	165 360

EXAMPLE 6

Effect of lipase dosage on washing

This example shows the influence of dosage of H. lanuginosa lipase on washing performance, using acetone fractionated lipase powder from DSM 3819.

Acetone fractionation was done on 10g of the crude powder prepared in Example 1, dissolved in water to 104 ml total volume. Final acetone concentration was 45% by volume. After freeze-drying the re-dissolved acetone precipitate, 0.629 g with an activity of 160,960 LU/g was obtained. This was used in the following washing tests.

Detergent composition: Washing time Temperature. pH (initial): Soiling:	LAS (0.5 g/l), Na_2CO_3 (1.0 g/l) 20 min 30°C 9.5 85 μl olive oil

The soiled swatches used were a different batch from Examples 2 - 5, so results are not directly comparable.

Lipase dosage, LU/ml	0	500	1500	3000	6000	10,000
Residual oil (mg) n (μ mole)	232 761	209 558	202 521	202 471	194 437	176 363

EXAMPLE 7

Comparison of Humicola lipases in washing

This example compares the washing effect obtained with lipase from H. lanuginosa (DSM 4109), H. brevis var. thermoidea (DSM 4111), H. brevispora (DSM 4110) and H. insolens (DSM 1800).

Detergent composition:	LAS Tallow soap Alcoholethoxylate (C_{12-14},6EO) Alcoholethoxylate (C_{16-18},30EO) Zeolite Na_2CO_3 Sodium metasilicate EDTA (titriplex III) Na_2SO_4	0.50 g/l 0.05 - 0.10 - 0.02 - 1.20 - 0.50 - 0.10 - 0.01 - 2.00 -
Temperature: Washing time: Lipase dosage: pH: Soiling:	30°C 20 min 6000 LU/l 9.5 50µl olive oil)	

Lipase preparation	n (µmoles)
none	546
H. lanuginosa	454
H. brevis var. thermoidea	468
H. brevispora	484
H. insolens	350

EXAMPLE 8

Protease stability of Humicola lipases

The excellent stability of Humicola lipases in detergent solutions containing proteolytic enzymes is demonstrated below.

A Humicola lanuginosa preparation (DSM 4109) is compared to the Fusarium oxysporum lipase used in previous examples and to the commercial lipase preparation, Amano P (Amano Pharmaceutical co. Ltd., Nagoya, Japan), which is produced by Pseudomonas fluorescens.

The alkaline Bacillus proteases ALCALASE, SAVINASE and ESPERASE were used. These are commercial detergent proteases from Novo Industri A/S, Denmark.

The proteolytic activity was determined with casein as the substrate. One Casein Protease Unit (CPU) is defined as the amount of enzyme liberating 1 mM of primary amino groups (determined by comparison with a serine standard) per minute under standard conditions, i.e. incubation for 30 minutes at 25°C and pH 9.5. A 2% (w/v) solution of casein (Hammersten, supplied by Merck A.G., West Germany) was prepared with the Universal Buffer described by Britton and Robinson (Journ. Chem. Soc. 1931, p. 1451) adjusted to pH 9.5.

Detergent:	1.3 g/l of a non-phosphate powder containing 25% surfactant (alpha-olefin sulphonate (AOS) and linear alkyl-benzene sulphonate (LAS)), sodium sulphate, zeolite, sodium silicate and optical brightener.
Water hardness:	4.5° German hardness
pH:	10.0 (adjusted)
Temperature:	25°C
Lipase activity (initial):	3000 LU/l
Protease activity:	0 or 0.05 CPU/l
Residual lipase activity (%):	

1) Protease: SAVINASE

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	99	94	91	89
Fusarium oxysporum	100	32	14	3	-
Pseudomonas fluorescens	100	1	0	-	-

2) Protease: None

Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	101	99	102	96
Fusarium oxysporum	100	57	42	18	6
Pseudomonas fluorescens	100	94	94	88	90

Detergent:	LAS Alcoholethoxylate (Berol 065) Tallow soap Sodium tripolyphosphate Sodium metasilicate CMC Na_2SO_4	0.40 g/l 0.15 g/l 0.15 g/l 1.50 g/l 0.40 g/l 0.05 g/l 2,10 g/l
Water hardness: pH: Temperature: Lipase activity: Protease activity:	18° German hardness 9.5 30°C 3000 LU/l 0 or 0.05 CPU/l	

1) Protease: SAVINASE					
Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	99	98	97	97
Fusarium oxysporum	100	5	0	-	-
Pseudomonas fluorescens	100	0	-	-	-

2) Protease: ALCALASE					
Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	98	97	95	95
Fusarium oxysporum	100	24	4	0	-
Pseudomonas fluorescens	100	18	0	-	-

3) Protease: ESPERASE					
Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	97	96	96	98
Fusarium oxysporum	100	20	0	-	-
Pseudomonas fluorescens	100	0	-	-	-

4) Protease: None					
Lipase	Incubation time (min)				
	0	15	30	60	90
Humicola lanuginosa	100	96	95	96	94
Fusarium oxysporum	100	30	10	0	-
Pseudomonas fluorescens	100	101	102	102	99

It is seen that the Humicola lipase of the invention is very stable in detergent solution with protease, in contrast to prior-art detergent lipases (Fusarium and Pseudomonas).

EXAMPLE 9

Stabilized liquid Humicola lipase preparations

Lipase stability in solutions with various stabilizers was investigated.

Lipase:	Humicola lanuginosa (DSM 4109)
Storage temp:	30 °C
pH:	7.0

Rodalon™ was added to all preparations as a preservative (0.2 mg active matter per ml).

Results:

	1,2-propanediol (ml/ml)	Sorbitol (g/ml)	$CaCl_2.2H_2O$ (mg/ml)	INITITAL ACTIVITY (LU/ml)	Residual activity (%), days 0	2	13	29	49
1	0	0	0	4520	100	17	2	< 1	< 1
2	0.50	0	0	4520	100	93	63	35	34
3	0.50	0	3	4720	100	86	76	54	48
4	0	0.30	0	4880	100	57	10	< 1	< 1

These results demonstrate that 1,2-propanediol (MPG = mono propylene glycol) is an excellent stabilizer for Humicola lipase. The storage stability may be further improved by adding Ca salt. Sorbitol has a slight stabilizing effect.

A wash trial was carried out with a stabilized liquid lipase preparation with the following composition:

Humicola lanuginosa DSM 4109 lipase	5000 LU/ml
1,2-propanediol	50% v/v
Deionized water	50% v/v
$CaCl_2.2H_2O$	3 mg/ml

Detergent composition:	LAS (0.5 g/l), Na_2CO_3 (1.0 g/l)
Temperature:	30 °C
Washing time:	20 minutes
pH:	9.5
Soiling:	50 µl olive oil
Lipase dosage:	1 ml stabilized liquid preparation per liter wash liquor.

Results:

Lipase dosage LU/l	Residual oil (mg)	n (µmoles)
0	157	507
5000	145	418

EXAMPLE 10

Humicola lipase as dust-free granulate

A carrier granulate without enzyme was prepared essentially according to US patent 4,106,991 with the following composition:

15% cellulose fibres
4% titanium dioxide
5% yellow dextrin
10% sucrose
64% sodium sulphate

This granulate was sieved to obtain a 300-710 µm particle size.

30.8 g of this was wetted by 6.2 g of a 30% solution in ethanol of polyvinylpyrrolidon (PVP K30, product of GAF Corp., USA). After thorough mixing, 12.3 of freeze-dried H. lanuginosa DSM 4109 lipase (92,700 LU/g, prepared essentially as in Example 2) was added, and was thoroughly mixed. The granulate was dried (evaporation of ethanol) by air blowing (fluidization) at about 50 °C. 300-850 µm particles were collected by sieving.

The granulate was then coated in three steps, as follows:

- 5% of polyethyleneglycol (MW 600)
- 11.25% of TiO_2/Mg silicate (4:1)
- 4% of polyethyleneglycol (MW 600)

The coated granulate was air-blown at 0.8 m/sec for 10 minutes to remove fine particles of coating material. Finally, the material was sieved again, and the 300-850 µm range was collected. A dust-free, off-white granulate was obtained.

Yield and activity were as follows:

freeze-dried powder	92,700 LU/g	12.3 g
un-coated granulate	21,100 -	45.0 -
coated granulate	18,200 -	

A washing trial was carried out with the freeze-dried powder and the granulate as follows:

Detergent composition: Temperature: Washing time: Lipase dosage: pH: Soiling:	LAS (0.5 g/l), Na_2CO_3 (1.0 g/l) 30°C 20 minutes 6000 LU/l 10.0 50 µl olive oil

Results:

Lipase preparation	n (µmoles)
none	515
freeze-dried powder	386
granulate	415

Claims

1. An enzymatic detergent additive, which contains, as active components, a microbially produced lipase and a proteolytic enzyme wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic activity is between about 0.5 and about 3.0 ANSON units/g of additive.

2. An enzymatic detergent additive, which contains, as active components, a microbially produced lipase and a proteolytic enzyme, wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic enzyme is an alkaline Bacillus protease.

3. The enzymatic detergent additive according to Claim 2, wherein the proteolytic enzyme is manufactured microbially from Bacillus licheniformis or is manufactured according to US Patent No. 3,723,250.

4. The enzymatic detergent additive according to Claim 1 or 2, wherein the lipase cross-reacts immunologically with a lipase from Humicola lanuginosa strain DSM 3819.

5. The enzymatic detergent additive according to any of claims 1 - 4, wherein the Humicola sp. is selected from H. lanuginosa (Griffon and Maublanc) Bunce (also described under the synonyms Thermomyces lanuginosus Tsiklinsky, Sepeclonium lanuginosum Griffon and Maublanc, Sepeclonium thermaphilum cyclosporum and S. thermaphilum ovosporum Velich, Acremoniella sp. Rege, Arcremoniella thermophila Curzi, and Monotospora lanuginosa (Griffon and Maublanc) Mason), H. brevispora Subrahmanyam and Thirumalachar, H. brevis var. thermoidea Subrahmanyam and Thirumalachar and H. insolens Cooney & Emerson.

6. The enzymatic detergent additive according to Claim 5, wherein the strain is H. lanuginosa DSM 3819.

7. The enzymatic detergent additive according to any of claims 1 - 6, wherein the additive is provided as a non-dusting granulate or a liquid.

8. The enzymatic detergent additive according to any of claims 1 - 6, wherein the additive is provided as a liquid containing an enzyme stabilizer.

9. The enzymatic detergent additive according to Claim 8, wherein the stabilizer is propylene glycol.

10. The enzymatic detergent additive according to any of claims 1 - 8, wherein the lipase activity is above about 10,000 LU/g of additive (1 LU (Lipase Unit) being the amount of enzyme which liberates 1 µmol titratable butyric acid from tributyrin per minute at 30°C, pH 7.0, with gum arabic as an emulsifier).

11. The enzymatic detergent additive according to Claim 1, wherein the proteolytic enzyme is an alkaline Bacillus protease.

12. The enzymatic detergent additive according to Claim 11, wherein the proteolytic enzyme is manufactured microbially by means of Bacillus licheniformis or is manufactured according to US Patent No. 3,723,250.

13. A detergent composition comprising a microbially produced lipase and a proteolytic enzyme wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic activity is between about 0.0005 and 0.15 ANSON units/g of detergent.

14. The detergent composition according to Claim 13, wherein the lipase cross-reacts immunologically with a lipase from Humicola lanuginosa strain DSM 3819.

15. A detergent composition comprising a microbially produced lipase and a proteolytic enzyme, wherein the lipase cross-reacts immunologically with a lipase produced by a Humicola sp., and wherein the proteolytic enzyme is an alkaline Bacillus protease.

16. The detergent composition according to Claim 15, wherein the proteolytic enzyme is manufactured microbially from Bacillus licheniformis or is manufactured according to US Patent No. 3,723,250.

17. The detergent composition according to Claim 15 or 16, wherein the lipase cross-reacts immunologically with a lipase from Humicola lanuginosa strain DSM 3819.

18. A detergent composition comprising the enzymatic detergent additive according to any of claims 1 - 12.

19. The detergent composition according to Claim 18, wherein the detergent contains the enzymatic detergent additive according to any of claims 1 - 12 in an amount of between 0.1 and 5% w/w.

20. The detergent composition according to any of claims 13 - 19 provided as a powder or liquid.

21. The detergent composition according to any of claims 13 - 20, wherein the surface active material comprises from 30 to 100 weight % of anionic surfactant and from 0 to 70 weight % of nonionic surfactant.

22. The detergent composition according to any of claims 13 - 21, wherein the lipase activity is between 50 and 20,000 LU/g of detergent (1 LU (Lipase Unit) being the amount of enzyme which liberates 1 μmol titratable butyric acid from tributyrin per minute at 30°C, pH 7.0, with gum arabic as an emulsifier).

23. The detergent composition according to any of claims 15 - 22, wherein the protease activity is between 0.0005 and 0.15 ANSON units/g of detergent.

24. A washing process using the detergent composition according to any of claims 13 - 23 at a pH between 7 and 12.

25. The washing process according to Claim 24, wherein the washing solution contains the detergent according to any of claims 13 - 23 in an amount of between 0.5 and 20 g per litre of washing solution.

Patentansprüche

1. Ein enzymhaltiger Reinigungsmittelzusatz, der als wirksame Bestandteile eine mikrobiell produzierte Lipase und ein proteolytisches Enzym enthält, wobei die Lipase immunologisch mit einer von einem Humicola sp. produzierten Lipase kreuzreagiert und wobei die proteolytische Aktivität zwischen etwa 0,5 und etwa 3,0 ANSON-Einheiten/g Zusatz liegt.

2. Ein enzymhaltiger Reinigungsmittelzusatz, der als wirksame Bestandteile eine mikrobiell produzierte Lipase und ein proteolytisches Enzym enthält, wobei die Lipase immunologisch mit einer von einem

Humicola sp. produzierten Lipase kreuzreagiert und wobei das proteolytische Enzym eine alkalische Bacillus-Protease ist.

3. Der enzymnaltige Reinigungsmittelzusatz nach Anspruch 2, wobei das proteolytische Enzym mikrobiell aus Bacillus licheniformis hergestellt ist oder gemäß US-Patent Nr. 3,723,250 hergestellt ist.

4. Der enzymhaltige Reinigungsmittelzusatz nach Anspruch 1 oder 2, wobei die Lipase immunologisch mit einer Lipase aus Humicola lanuginosa-Stamm DSM 3819 kreuzreagiert.

5. Der enzymhaltige Reinigungsmittelzusatz nach einem der Ansprüche 1-4, wobei der Humicola sp. ausgewählt ist aus H. lanuginosa (Griffon und Maublanc) Bunce (auch beschrieben unter den Synonymen Thermomyces lanuginosus Tsiklinsky, Sepeclonium lanuginosum Griffon und Maublanc, Sepeclonium thermaphilum cyclosporum und S. thermaphilum ovosporum Velich, Acremoniella sp. Rege, Acremoniella thermophila Curzi und Montospora lanuginosa (Griffon und Maublanc) Mason), H. brevispora Subrahmanyam und Thirumalachar, H. brevis var. thermoidea Subrahmanyam und Thirumalachar und H. insolens Cooney & Emerson.

6. Der enzymatische Reinigungsmittelzusatz nach Anspruch 5, wobei der Stamm H. lanuginosa DSM 3819 ist.

7. Der enzymatische Reinigungsmittelzusatz nach einem der Ansprüche 1-6, wobei das Additiv als ein nicht-staubendes Granulat oder eine Flüssigkeit bereitgestellt ist.

8. Der enzymatische Reinigungsmittelzusatz nach einem der Ansprüche 1-6, wobei der Zusatz als eine Flüssigkeit bereitgestellt ist, die einen Enzymstabilisator enthält.

9. Der enzymatische Reinigungsmittelzusatz nach Anspruch 8, wobei der Stabilisator Propylenglykol ist.

10. Der enzymatische Reinigungsmittelzusatz nach einem der Ansprüche 1-8, wobei die Lipaseaktivität über etwa 10.000 LU/g Zusatz liegt (wobei 1 LU (Lipase Unit) die Enzymmenge ist, die 1 μmol titrierbare Buttersäure aus Tributyrin pro Minute bei 30 °C, pH 7,0, mit Gummi arabicum als einem Emulgator freisetzt).

11. Der enzymhaltige Reinigungsmittelzusatz nach Anspruch 1, wobei das proteolytische Enzym eine alkalische Bacillus-Protease ist.

12. Der enzymhaltige Reinigungsmittelzusatz nach Anspruch 11, wobei das proteolytische Enzym mikrobiell mit Hilfe von Bacillus licheniformis hergestellt ist oder gemäß US-Patent Nr. 3,723,250 hergestellt ist.

13. Eine Reinigungsmittelzusammensetzung, die eine mikrobiell produzierte Lipase und ein proteolytisches Enzym umfaßt, wobei die Lipase immunologisch mit einer von einen Humicola sp. produzierten Lipase kreuzreagiert und wobei die proteolytische Aktivität zwischen etwa 0,0005 und 0,15 ANSON-Einheiten/g Reinigungsmittel liegt.

14. Die Reinigungsmittelzusammensetzung nach Anspruch 13, wobei die Lipase immunologisch mit einer Lipase aus Humicola lanuginosa-Stamm DSM 3819 kreuzreagiert.

15. Eine Reinigungsmittelzusammensetzung, die eine mikrobiell produzierte Lipase und ein proteolytisches Enzym umfaßt, wobei die Lipase immunologisch mit einer von einem Humicola sp. produzierten Lipase kreuzreagiert und wobei das proteolytische Enzym eine alkalische Bacillus-Protease ist.

16. Die Reinigungsmittelzusammensetzung nach Anspruch 15, wobei das proteolytische Enzym mikrobiell aus Bacillus licheniformis hergestellt ist oder gemäß US-Patent Nr. 3,723,250 hergestellt ist.

17. Die Reinigungsmittelzusammensetzung nach Anspruch 15 oder 16, wobei die Lipase immunologisch mit einer Lipase aus Humicola lanuginosa-Stamm DSM 3819 kreuzreagiert.

18. Eine Reinigungsmittelzusammensetzung, die den enzymhaltigen Reinigungsmittelzusatz nach einem der Ansprüche 1-12 umfaßt.

19. Die Reinigungsmittelzusammensetzung nach Anspruch 18, wobei das Reinigungsmittel den enzymhaltigen Reinigungsmittelzusatz nach einem der Ansprüche 1-12 in einer Menge zwischen 0,1 und 5 Gew.-% enthält.

20. Die Reinigungsmittelzusammensetzung nach einem der Ansprüche 13-19, bereitgestellt als ein Pulver oder eine Flüssigkeit.

21. Die Reinigungsmittelzusammensetzung nach einem der Ansprüche 13-20, wobei das oberflächenaktive Material von 30 bis 100 Gewichts-% anionisches Tensid und 0 bis 70 Gewicht-% nicht-ionisches Tensid umfaßt.

22. Die Reinigungsmittelzusammensetzung nach einem der Ansprüche 13-21, wobei die Lipaseaktivität zwischen 50 und 20.000 LU/g Reinigungsmittel liegt (wobei 1 LU (Lipase Unit) die Enzymmenge ist, die 1 µmol titrierbare Buttersäure aus Tributyrin pro Minute bei 30°C, pH 7,0, mit Gummi arabicum als einem Emulgator freisetzt).

23. Die Reinigungsmittelzusammensetzung nach einem der Ansprüche 15-22, wobei die Proteaseaktivität zwischen 0,0005 und 0,15 ANSON-Einheiten/g Reinigungsmittel liegt.

24. Ein Waschverfahren unter Verwendung der Reinigungsmittelzusammensetzung nach einem der Ansprüche 13-23 bei einen pH zwischen 7 und 12.

25. Das Waschverfahren nach Anspruch 24, wobei die Waschlösung das Reinigungsmittel nach einem der Ansprüche 13-23 in einer Menge von zwischen 0,5 und 20 g pro Liter Waschlösung enthält.

Revendications

1. Additif enzymatique pour détergent qui contient, en tant que composants actifs, une lipase produite par voie microbienne et une enzyme protéolytique, dans lequel la lipase entre en réaction croisée immunologiquement avec une lipase produite par Humicola sp., et dans lequel l'activité protéolytique se situe entre environ 0,5 et environ 3,0 unités ANSON/g d'additif.

2. Additif enzymatique pour détergent qui contient, en tant que composants actifs, une lipase produite par voie microbienne et une enzyme protéolytique, dans lequel la lipase entre en réaction croisée immunologiquement avec une lipase produite par Humicola sp., et dans lequel l'enzyme protéolytique est une protéase alcaline de Bacillus.

3. Additif enzymatique pour détergent selon la revendication 2, dans lequel l'enzyme protéolytique est fabriquée par voie microbienne à partir de Bacillus licheniformis ou est fabriquée selon le brevet US n° 3 723 250.

4. Additif enzymatique pour détergent selon la revendication 1 ou 2, dans lequel la lipase entre en réaction croisée immunologiquement avec une lipase provenant d'une souche Humicola lanuginosa DSM 3819.

5. Additif enzymatique pour détergent selon l'une quelconque des revendications 1-4 dans lequel l'Humicola sp. est choisie à partir d'H. lanuginosa (Griffon and Maublanc) Bunce (également décrite sous les synonymes de Thermomyces lanuginosus Tsiklonsky, Sepeclonium lanuginosum Griffon and Maublanc, Sepeclonium thermaphilum cyclosporum et S. thermaphilum ovosporum Velich, Acremoniella sp. Rege, Arcremoniella thermophila Curzi, et Monotospora lanuginosa (Griffon and Maublanc) Mason, H. brevispora Subrahmanyam and Thirumalachar, H. brevis var. thermoidea Subrahmanyam and Thirumalachar et H. insolens Cooney & Emerson.

6. Additif enzymatique pour détergent selon la revendication 5, dans lequel la souche est la H. lanuginosa DSM 3819.

7. Additif enzymatique pour détergent selon l'une quelconque des revendications 1 à 6, dans lequel l'additif se présente sous la forme de granulés ne produisant pas de poussière ou sous forme de liquide.

8. Additif enzymatique pour détergent selon l'une quelconque des revendications 1 à 6, dans lequel l'additif se présente sous la forme de liquide contenant un stabilisant enzymatique.

9. Additif enzymatique pour détergent selon la revendication 8, dans lequel le stabilisant est le propylène-glycol.

10. Additif enzymatique pour détergent selon l'une quelconque des revendications 1 à 8, dans lequel l'activité lipase se situe au-dessus de 10 000 UL/g d'additif (1 UL (Unité Lipase) étant la quantité d'enzymes qui libère 1 µmol titrable d'acide butyrique à partir de tributyrine par minute à 30 °C, pH 7,0, avec de la gomme arabique comme émulsifiant).

11. Additif enzymatique pour détergent selon la revendication 1, dans lequel l'enzyme protéolytique est une protéase alcaline de Bacillus.

12. Additif enzymatique pour détergent selon la revendication 11, dans lequel l'enzyme protéolytique est fabriquée par voie microbienne aux moyens de Bacillus licheniformis ou est fabriquée selon le brevet US n° 3 723 250.

13. Composition de détergent comprenant une lipase produite par voie microbienne et une enzyme protéolytique, dans laquelle la lipase entre en réaction croisée immunologiquement avec une lipase produite par une Humicola sp., et dans laquelle l'activité protéolytique se situe entre environ 0,0005 et 0,15 unités ANSON/g de détergent.

14. Composition de détergent selon la revendication 13, dans laquelle la lipase entre en réaction croisée immunologiquement avec une lipase à partir d'une souche Humicola lanuginosa DSM 3819.

15. Composition de détergent comprenant une lipase produite par voie microbienne et une enzyme protéolytique, dans laquelle la lipase entre en réaction croisée immunologiquement avec une lipase produite par voie Humicola sp., et dans laquelle l'enzyme protéolytique est une protéase alcaline de Bacillus.

16. Composition de détergent selon la revendication 15, dans laquelle l'enzyme protéolytique est fabriquée par voie microbienne à partir de Bacillus licheniformis ou est fabriquée selon le brevet US n° 3 723 250.

17. Composition de détergent selon la revendication 15 ou 16, dans laquelle la lipase entre en réaction croisée immunologiquement avec une lipase de la souche Humicola lanuginosa DSM 3819.

18. Composition de détergent comprenant l'additif enzymatique pour détergent selon l'une quelconque des revendications 1 à 12.

19. Composition de détergent selon la revendication 18, dans laquelle le détergent contient l'additif enzymatique pour détergent selon l'une quelconque des revendications 1 à 12 dans une quantité entre 0,1 et 5% poids/poids.

20. Composition de détergent selon l'une quelconque des revendications 13 à 19, prévue sous forme de poudre ou de liquide.

21. Composition pour détergent selon l'une quelconque des revendications 13 à 20, dans laquelle le matériau tensioactif comprend de 30 à 100% en poids d'agent tensioactif anionique et de 0 à 70 % en poids d'agent tensioactif non ionique.

22. Composition pour détergent selon l'une quelconque des revendications 13 à 21, dans laquelle l'activité lipase se situe entre 50 et 20 000 UL/g de détergent (1 UL (Unité Lipase) étant la quantité d'enzymes

qui libère 1 µmol d'acide butyrique titrable à partir de tributyrine par minute à 30 °C, pH 7,, avec de la gomme arabique comme émulsifiant).

23. Composition pour détergent selon l'une quelconque des revendications 15 à 22, dans laquelle l'activité protéase se situe entre 0,0005 et 0,15 unités ANSON/g de détergent.

24. Procédé de nettoyage utilisant la composition de détergent selon l'une quelconque des revendications 13 à 23, à un pH entre 7 et 12.

25. Procédé de lavage selon la revendication 24, dans lequel la solution de lavage contient le détergent selon l'une quelconque des revendications 13 à 23, dans une quantité entre 0,5 et 20 g par litre de solution de lavage.

Fig.1

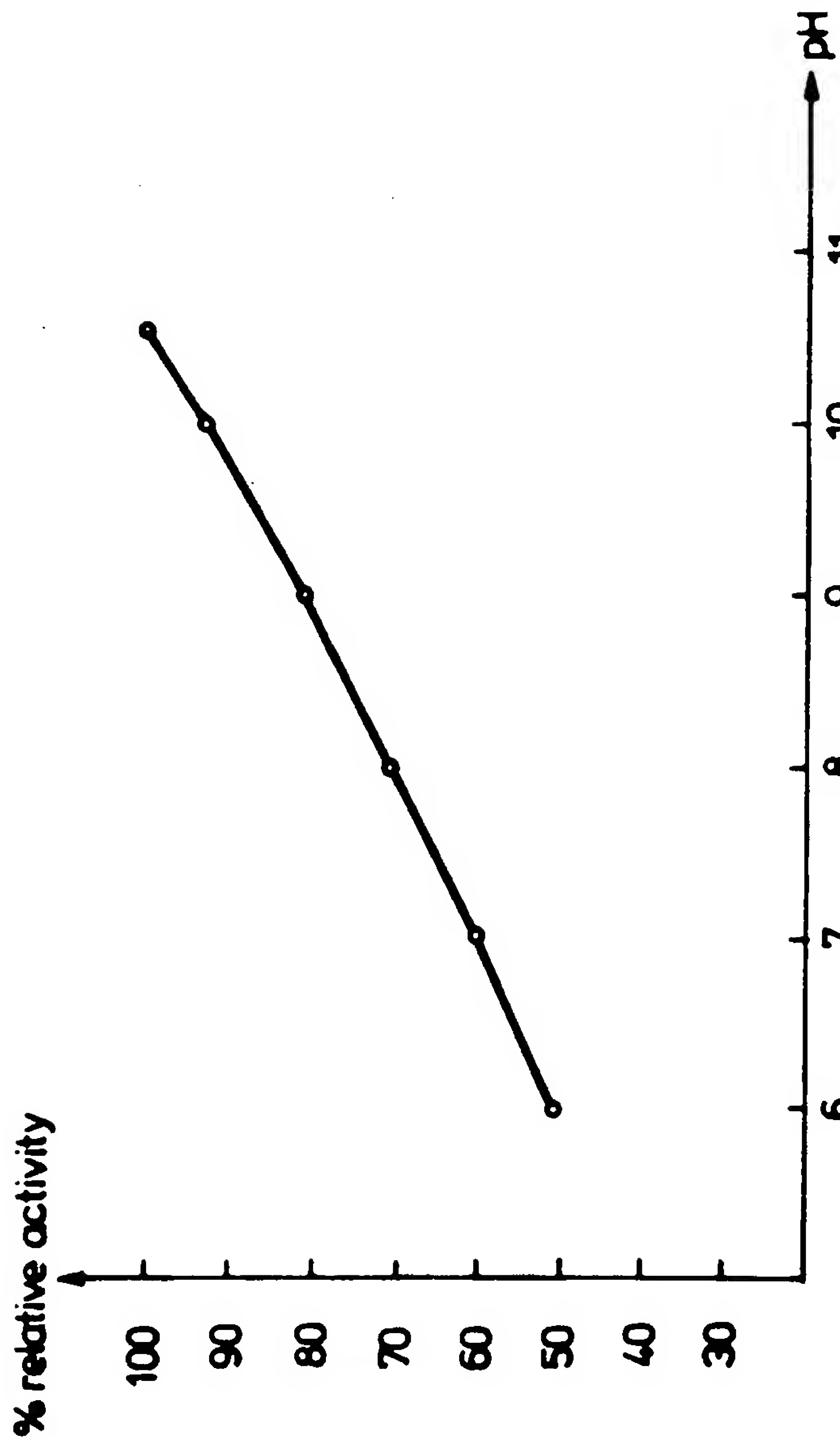

Fig. 2

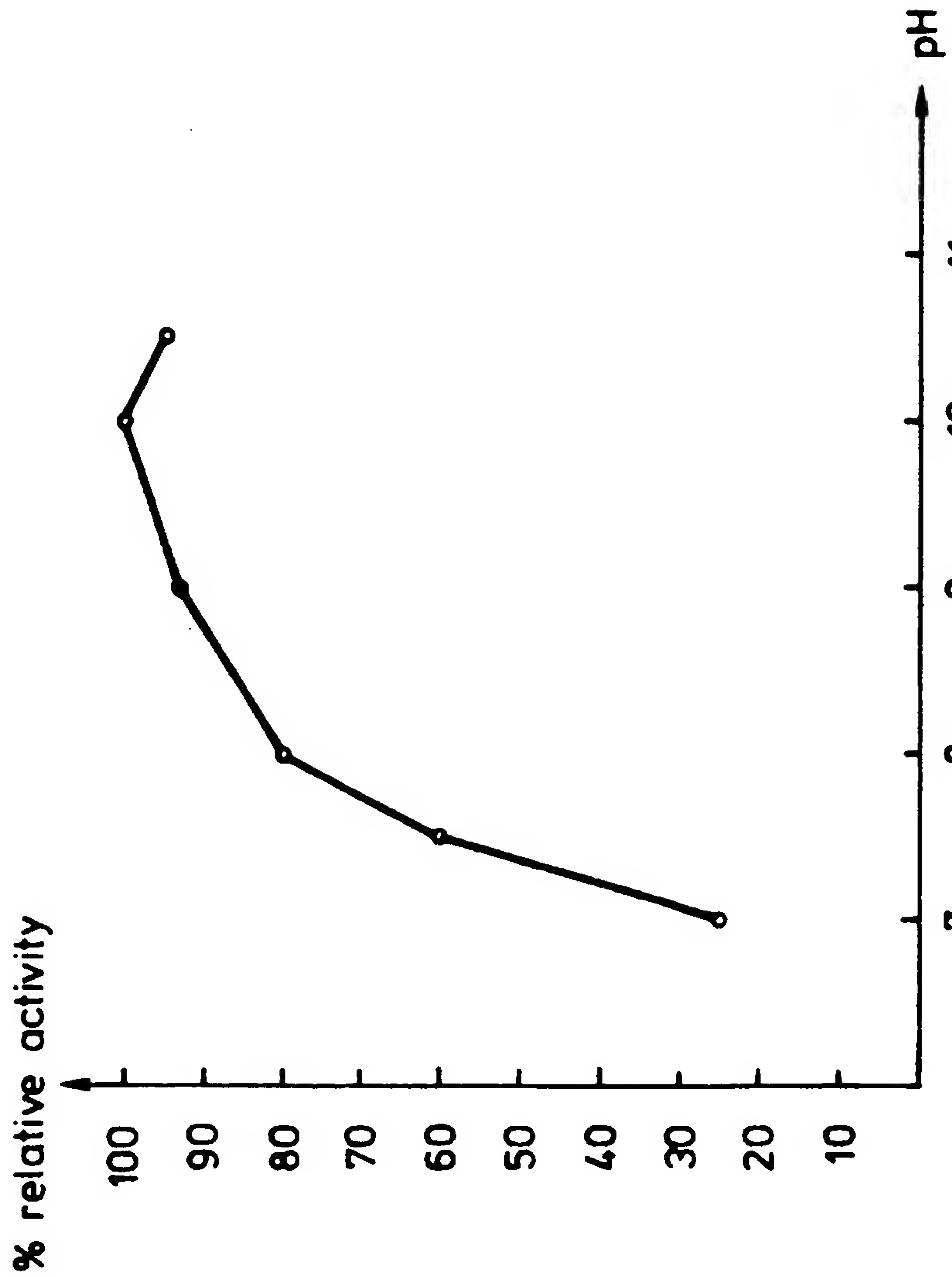

Fig. 3

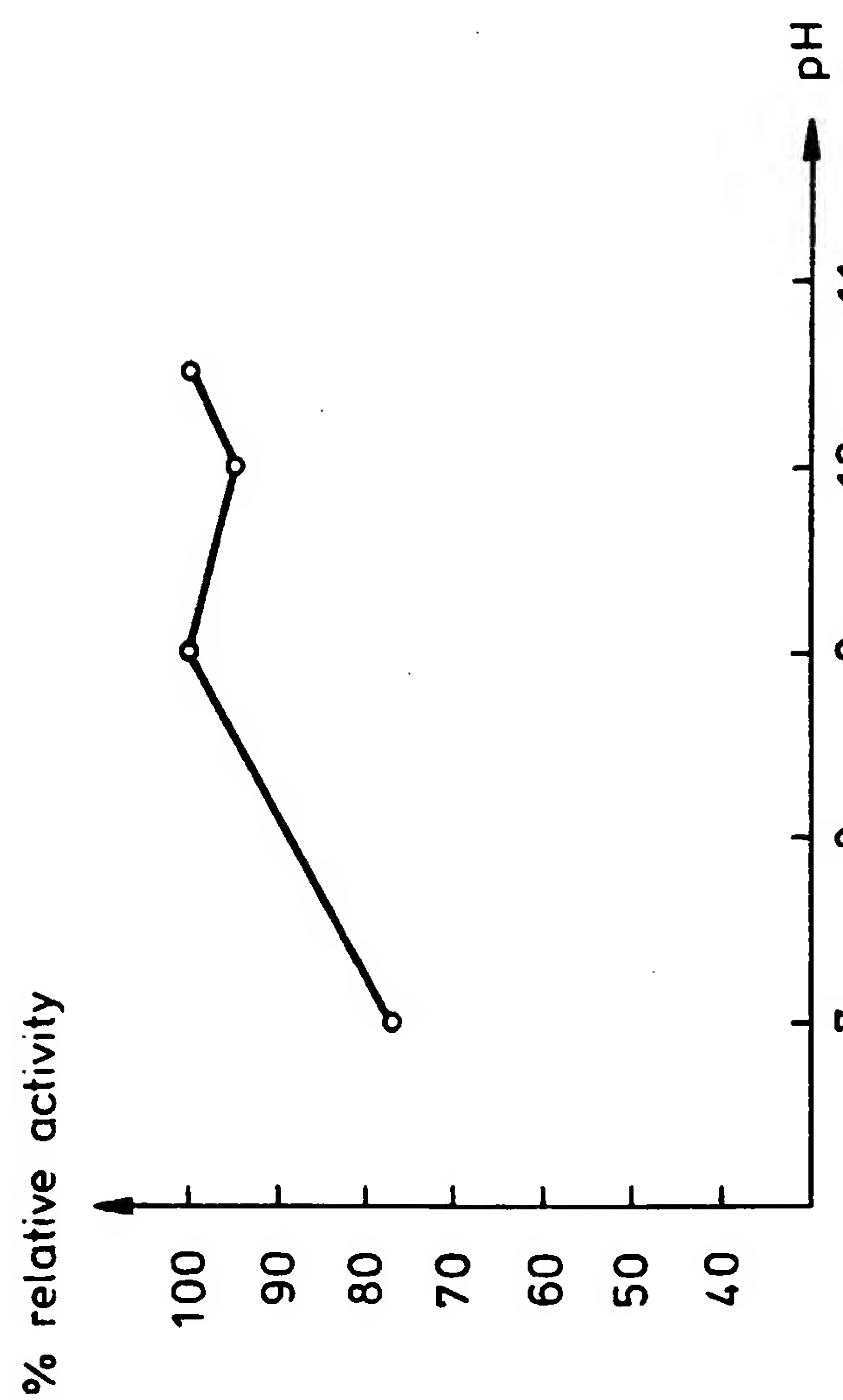

Fig. 4